
POLLINATOR EFFECTIVENESS ON CO-OCCURRING MILKWEEDS (*ASCLEPIAS*; APOCYNACEAE, ASCLEPIADOIDEAE)¹

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ABSTRACT

Plant-pollinator interactions constitute one of the most widely recognized mutualisms, yet most investigations focus on single species or specialized pollinators. We used multiple measures to evaluate the efficiency and effectiveness of a diverse assemblage of pollinators on three co-flowering species: *Asclepias syriaca* L., *A. incarnata* L., and *A. verticillata* L. Hymenopterans exceeded other insects in their prevalence as major pollinators, but most did not vary significantly among plant species in the number of flowers visited or in visit duration per plant, except for native *Bombus griseocollis*. Significant variation in visit duration was also uncommon among insects foraging on a single taxon, except for *Apis mellifera*, which foraged longer on plants of *A. syriaca* than native hymenopterans and lepidopterans. Among the hymenoptera, bumblebees, carpenter bees, and wasps visited more plants per foraging bout, respectively, on *A. syriaca*, *A. incarnata*, and *A. verticillata*. Insect fidelity also varied seasonally and among plant species. Thus, honeybees, lepidopterans, and sphecid wasps foraged with greater relative constancy on *A. syriaca*, *A. incarnata*, and *A. verticillata*, respectively. All bees and wasps carried more pollinaria of *A. incarnata* and *A. verticillata* than of the larger-flowered *A. syriaca*, but most insects had higher inferred pollinium transfer rates on *A. syriaca* than on its congeners, especially sphecid wasps. Overall, the actions of pollinators were individualistic and indices of pollinator effectiveness based on pre-contact foraging versus vector pollinium loads were not strongly congruent. Our results highlight the need for new and creative approaches to studying the role of generalized pollination systems in the origin and maintenance of sympatrically flowering species.

Key words: *Asclepias*, common garden, foraging behavior, milkweed, pollinator effectiveness, pollinator efficiency.

Plant-pollinator interactions are widely recognized mutualisms with the potential to shape the evolution of morphological and behavioral traits. The intensity of any reciprocal adaptation between organisms will be influenced by both “quantity” and “quality” components of the interaction (Herrera, 1989); i.e., by the frequency with which a given pollinator interacts with a plant species, multiplied by its impact on fitness. To compare the relative importance of pollinators, evolutionary ecologists have sought to standardize pollinator effectiveness and efficiency measures (Thomson & Thomson, 1992; Inouye et al., 1994; Dafni & Jürgens, unpubl. data). Although researchers often estimate effectiveness of individual pollinators based on pollen transfer or fruit-set following a single visit (Inouye et al., 1994; Ivey et al., 2003; Gross, 2005), the congruence of various measures of effectiveness remains largely untested. In addition, rarely are these parameters estimated simultaneously for co-occurring plants that potentially compete for pollinator services (e.g., Dafni et al., 1997; Le Corff et al., 1998; McIntosh, 2005).

Ideally, comparative measures of pollinator effectiveness for co-occurring plant taxa would incorporate frequencies of pollinator visits, as well as data on pollen loads, patterns of pollen transfer, relative rates of pollen removal and deposition, and contributions to fruit and seed set. Pollinators that rarely visit or those with large pollen loads but little success at delivery may be less effective than pollinators with moderate visitation rates, but efficient pollen transfer (Thomson & Thomson, 1992). Behavior that leads to geitonogamy can also waste pollen resources in self-incompatible plants or reduce the effectiveness of pollination where heterozygote advantage exists (e.g., Broyles & Wyatt, 1993; Harder & Barrett, 1995). Such ineffective pollination may arise in part from inherent conflicts between behaviors that benefit the plant versus those that benefit the pollinator (Herre et al., 1999; Gregear & Lavery, 2001). Floral specialization or specialist foragers can enhance pollen transfer, but many plants are visited by generalist pollinators (Waser et al., 1996; Johnson & Steiner, 2000) or may share similar “floral syndromes” with

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Figure 1. Pollinarium structure and major pollinators visiting three species of *Asclepias*. —A. Pollinarium of *A. syriaca* attached to corpusculum of *A. verticillata* and insect claw. —B. *Sphex pennsylvanicus* visiting *A. incarnata*. —C. *Xylocopa virginica* visiting *A. verticillata*. —D. *Bombus griseocollis* visiting *A. syriaca*.

close congeners, which might lead to partitioning of pollinator resources (Kephart, 1983).

Milkweeds (*Asclepias* L.) are particularly amenable to analyses of pollinator behavior and effectiveness in sympatric species. They are prevalent in North America, where more than 100 species with overlapping distributions are recognized (Woodson, 1954; Fishbein, 2001). The unusual flowers vary in floral architecture, yet attract diverse “generalist” visitors (Robertson, 1929; Fishbein & Venable, 1996; Ivey et al., 2003; Kephart & Theiss, 2004). The pollination system in *Asclepias* also facilitates evaluation of pollinator effectiveness. First, the pollen is packaged en masse in a pollinarium (i.e., a corpusculum joined

via translator arms to two pollinia; Fig. 1). Second, insects inadvertently remove pollinaria when their appendages engage grooves in the corpuscula positioned above each of five stigmatic chambers. Moreover, corpuscula are often retained for some time on insects after delivery of the pollinia. Thus, vector pollen loads and the proportions potentially transferred to flowers can be estimated from the number of corpuscula and pollinia remaining on insect body parts (e.g., Robertson, 1929; Kephart & Theiss, 2004).

We gathered data in natural communities and a common garden to evaluate the relative effectiveness of major hymenopteran and lepidopteran polli-

nators of three co-occurring species of *Asclepias*. We define and compare multiple measures of pollinator effectiveness, including the efficiency of pollen transfer, to address the following questions. (1) *Do pollinators vary in behavior and effectiveness on diverse species of milkweeds?* For example, the relative sizes of flowers and pollinators might affect both foraging behavior and the likelihood of pollen transfer. (2) *Does pollinator effectiveness vary seasonally?* We hypothesized that foraging time on flowers, per flower visitation rates, and pollinator fidelity might vary with the extent of flowering overlap. (3) *Do different measures of pollinator effectiveness produce similar rankings of pollinators on sympatric asclepiads?* If adaptation to different pollinators exists among plant species, we predicted that multiple measures of pollinator effectiveness would produce similar outcomes.

METHODS

STUDY SPECIES AND POPULATIONS

We used direct and indirect methods to compare the effectiveness of putative pollinators on three species of *Asclepias*: *A. syriaca* L., *A. incarnata* L., and *A. verticillata* L. These asclepiads are sympatric to parapatric in mesic prairies and disturbed sites. *Asclepias syriaca* is the most common, co-occurring near *A. incarnata* in moist areas and with *A. verticillata* in drier habitats (Woodson, 1954; Curtis, 1955). Plants flower from June to August, with variable overlap between species across sites and years, but *A. syriaca* flowers consistently ahead of its congeners (Kephart, 1987). Many of the same pollinators visit all three species (Kephart & Theiss, 2004), which differ in height, inflorescence architecture, flower size, and color (Fig. 1). The light rose to deep maroon flowers of *A. syriaca* and *A. incarnata* emit strong, sweet fragrances (e.g., linalool, *trans*- β -ocimene) that differ from the weak odor in *A. verticillata* (Dotterl et al., unpubl., Univ. Bayreuth). Plant vouchers are deposited at Chicago Botanic Garden (*A. syriaca*, Paterson 1480) and Morton Arboretum (*A. incarnata*, Curtis 63402 and *A. verticillata*, Swink, Betz & Lamp 7387).

We compared pollinators on plants in diverse natural populations across the geographic ranges of the species, but predominantly in Illinois and Indiana. We also observed species in experimental arrays of equal densities ($n = 18$ same-aged plants per plot), established in a common garden (Kephart & Theiss, 2004). Although *Asclepias incarnata* and *A. verticillata* flowered in the first and subsequent summers, most *A. syriaca* plants did not flower until the second year;

thus, we evaluated insect visitation to all three species in the second and third years of the study.

MEASURES OF POLLINATOR EFFECTIVENESS AND EFFICIENCY

Pollinator effectiveness is used broadly to characterize any one of various measures biologists use to compare the likelihood that a given pollinator will influence plant fitness (sensu Ivey et al., 2003; Gross, 2005). Recent studies exemplify a trend to retain the useful, but problematic descriptors of pollinator “efficiency” and “effectiveness,” while at the same time employing additional definitive terms (i.e., Inouye et al., 1994) for specific measurement variables (e.g., Engel & Irwin, 2003; Lau & Galloway, 2004; Gross, 2005). We computed multiple measures of pollinator effectiveness including visitation rate, time on flowers, constancy of visitation, and vector pollen load. We estimated pollen transfer efficiency based on insect capture data, as well as after single visits to virgin umbels by major pollinators, restricting the use of “efficiency” to ratios of variables that denote proportions of pollen transfer (i.e., Inouye et al., 1994). We defined potential pollinators as insects that consistently carried pollinaria and/or were observed to remove or insert pollinia.

POLLINATOR BEHAVIOR, VISITATION, AND CONSTANCY

We differentiated three seasonal periods for which flowering sequentially emphasized the largely June flowering *Asclepias syriaca* (“early” summer), relative to *A. verticillata* and *A. incarnata*, which vary in peak flowering from “mid” to “late” summer and typically cease flowering by late August. We used two methods to record behavior, including (1) 20-minute timed observations of all visitors in experimental arrays, with movements coded as between conspecific or heterospecific plants; and (2) “sequence observations” during which we followed and timed foragers in the order encountered until they left the area or foraged > 20 minutes on a plant. Variables recorded include the duration in seconds of each visit to an umbel, the number of flowers probed and plants visited of each species, and the sequence of plants visited during each foraging bout. Observations occurred throughout the day under clear and overcast skies, as long as visitors were active. Binoculars or close inspection were occasionally necessary to confirm the identity of the insect. Unknown insects were given descriptive names, and representatives were later captured outside the plot and/or compared to existing insect collections. In data on foraging bouts, *Bombus* refers largely to *B. griseocollis*, which constituted more than 98% of individuals observed in some years. We based

rankings of visitor abundances on *Asclepias* on these foraging bouts, as well as survey walks and transects and during 10–20-minute observation periods throughout the flowering season in natural populations and experimental garden plots (Ivey et al., 2003; Kephart & Theiss, 2004).

We computed visit duration per plant based on the time an insect worked the flowers of each plant during a foraging bout, excluding grooming and other non-foraging activities. We used Kruskal-Wallis and Mann-Whitney *U* tests to evaluate the effect of insect and plant taxon (independent variables) on the number of flower visits per plant and on visit duration. We also computed the proportion of each plant species represented in bouts with visits to more than one plant taxon. Values of dependent variables were averaged for each foraging bout prior to analysis to avoid pseudoreplication.

We modeled constancy (the proportion of movements between conspecific plants) using a generalized linear model, in which visitor taxon (“insect”), plant taxon from which movement originated (“plant”), insect $\times$ plant interaction, and “season” were fixed effects. We assigned binomial errors to the models and a logit linking function (Littell et al., 1996). The response was coded as either 1 (conspecific movement) or 0 (heterospecific movement). Because of small sample sizes, all visitors in Lepidoptera were combined into a single category for these analyses (“leps”). We included data from timed observation plots and from sequence observations in this analysis of constancy. We were unable to distinguish the contributions of individual foragers to the tallied interplant movements in some cases, so each movement was treated as an independent observation. However, the vast majority of insects visited ≤ 5 plants/bout (range 2–29); only 1.4% bouts exceeded 10 plants/bout.

POLLINATOR EFFECTIVENESS AND EFFICIENCY: CAPTURE DATA

We estimated pollinator effectiveness in removing pollinaria from flowers by counting the number of attached corpuscula, and their associated pollinia (0, 1, or 2), from insects collected haphazardly from *Asclepias* flowers in common garden and natural populations. This “Vector Pollen Load” (V_E ; sensu Inouye et al., 1994) estimates the total number of pollinia potentially removed and carried by major pollinators. Although V_E is affected by factors such as visitation rates, the time spent on flowers prior to capture, and grooming behavior, in some previous studies with milkweeds most hymenopterans and large lepidopteran pollinators with high pollinium loads also removed a high number of pollinaria during visits

to virgin umbels (Fishbein & Venable, 1996; but see Ivey et al., 2003). We compared the pollinium load of major pollinators across plant species using a Kruskal-Wallis test.

We estimated vector pollination efficiency (Inouye et al., 1994), i.e., the efficiency of pollen transfer from an insect’s body to a receptive stigma, by dividing the number of pollinia remaining on each captured insect by the total number of pollinaria on that insect. Subtracting this number from one gives an estimate of the proportion of the removed pollinia that were missing and presumably inserted by the insect. This estimate may not hold if recently attached pollinia are lost more readily or if insects carry chains of pollinaria (e.g., Morse, 1981) that may be lost en masse. However, this measure of pollination efficiency provides a point of comparison with estimates based on other methods, including those estimates derived from single visits of pollinators to virgin umbels.

All insects captured were identified to species and scored for the number and distribution of any attached pollinaria or corpuscula. We deposited insect vouchers for these specimens with Indiana University (IUC), University of Georgia (UGCA), and with United States Department of Agriculture (USDA) laboratories in Beltsville, Maryland, in exchange for full identifications.

SINGLE VISIT POLLINATOR EFFECTIVENESS

We examined pollinium removal and insertion following single visits to virgin umbels in natural populations in Virginia and Illinois over two field seasons. We pooled data across years and across sites, under the assumption that differences among insect species in effectiveness outweighed differences between sites or between years within species. We covered individual budding umbels with bags made of organza or featherweight pellon secured with plastic-coated wire. Once flowers had opened, we allowed a single insect to forage on each umbel and recorded the duration of each visit as well as the number of flowers visited. After the visit, we collected the umbel(s) and either froze the flowers or stored them in 70% ethanol. The flowers were later dissected, examined under a microscope, and scored for the number of pollinia removed and inserted.

RESULTS

POLLINATOR BEHAVIOR, VISITATION, AND CONSTANCY

The major pollinators observed during foraging bouts on asclepiads in common garden plots included three bees (*Bombus griseocollis*, *Apis mellifera*, *Xylocopa virginica*), two sphecids wasps (*Sphex*

pennsylvanicus, *S. ichneumoneus*), and two butterflies (*Danaus plexippus*, *Speyeria cybele*). However, visitation rates varied with both insect and plant taxa. For both years, the most common visitor, *B. griseocollis*, accounted for 44%–51% and 46%–57% of the foraging bouts on *Asclepias incarnata* and *A. syriaca*, respectively, but only 7%–19% of bouts on *A. verticillata*. *Apis mellifera* visited *A. syriaca* (20%–26% of bouts) more often than other asclepiads (6%–13% of bouts), whereas carpenter bees (*X. virginica*) found on *A. verticillata* and *A. incarnata* (e.g., 13%–17%, respectively, in year 3) were rare on *A. syriaca* (0%–2% of visits). Among lepidopterans, nymphalids and papilionids occurred often on *A. incarnata* and *A. syriaca* (up to 26% of observed visits). In contrast, butterflies were infrequent on *A. verticillata* (4%–5%) and included hesperids and pierids instead.

Insects differed somewhat in their behaviors on different plant taxa. The mean number of flower visits by a particular insect taxon varied significantly with plant species only for *Bombus griseocollis* ($P = 0.01$, $df = 2$, $N = 100$ bouts, $\chi^2 = 9.3$, Kruskal-Wallis), but all major native pollinators, i.e., *B. griseocollis*, *Sphex* species, and *Xylocopa virginica*, consistently visited more flowers while foraging on *Asclepias incarnata* than on co-flowering species (Fig. 2A). In addition, *Bombus* visited more plants/bout ($\mu = 5.6 \pm 0.5$) than other major visitors to *A. syriaca* ($P < 0.001$, $df = 4$, $N = 152$ bouts, $\chi^2 = 40$). On *A. incarnata*, papilionid and nymphalid butterflies, respectively, visited the most plants/bout ($\mu = 6.7\text{--}8.4 \pm 1\text{--}3$), although the mean for *X. virginica* (4.75 ± 0.8) significantly exceeded that for *A. mellifera*, *B. griseocollis*, both *Sphex* species, and *Danaus plexippus* ($P < 0.001$, $df = 7$, $N = 477$ bouts, $\chi^2 = 37$). In contrast, wasps visited more plants/bout ($\mu = 3.6\text{--}3.9 \pm 0.33\text{--}0.66$) than other foragers on *A. verticillata* ($P < 0.001$, $df = 5$, $N = 126$ bouts, $\chi^2 = 19$). The number of flower visits per plant did not differ among the major insect taxa foraging on any of the plant species (Fig. 2A; $P = 0.29\text{--}0.74$, Kruskal-Wallis), but sample sizes were low for bouts with complete data for this variable ($N = 3\text{--}58$ bouts).

Visit duration in seconds varied significantly among insects foraging on *Asclepias syriaca* ($P < 0.001$, $df = 7$, $N = 208$ bouts, $\chi^2 = 26$) and *A. incarnata* ($P = 0.02$, $df = 8$, $N = 241$ bouts, $\chi^2 = 18$; Fig. 2B) but not on *A. verticillata* ($P = 0.20$, $df = 6$, $N = 105$ bouts). Non-native *Apis mellifera* exhibited the highest residence times on *A. syriaca*, whereas visits to *A. incarnata* were longest for *Bombus griseocollis*, *Xylocopa virginica*, and *Danaus plexippus* (Fig. 2B). Visit duration for a given insect varied significantly among plant species only for the high duration of *A. mellifera* on *A. syriaca* versus *A. verticillata* ($P = 0.02$,

Mann-Whitney) and *B. griseocollis* on *A. incarnata* ($P = 0.03$, $df = 2$, $\chi^2 = 7.2$) relative to these same foragers on co-flowering plant congeners (Fig. 2B). For a given insect species, among-year differences also did not change the rank order among plant species; however, visits by *B. griseocollis* to *A. incarnata* and *A. syriaca* decreased in duration in the second study year (Mann-Whitney, $P = 0.001\text{--}0.04$) while visits by *Sphex ichneumoneus* to *A. incarnata* and *A. mellifera* to *A. verticillata* increased that same year ($P < 0.05$, Mann-Whitney).

The proportion of insect pollinator movements between conspecific plants in the common garden (“constancy”) varied temporally (“season”: $F_{2, 3919} = 78.43$, $P < 0.0001$); it was lowest early in the season (least squares mean $\pm$ standard error of the mean (SEM) = 0.66 ± 0.03), peaked mid-season (0.93 ± 0.01), and tapered somewhat toward the end of the season (0.83 ± 0.02), and all these values differed significantly from one another by Tukey-Kramer adjusted pairwise post-hoc t -tests ($P < 0.0001$). On average, there were no differences among plant taxa in constancy (“plant”: $F_{2, 3919} = 0.49$, $P = 0.6$), suggesting that the ability of a plant to inspire fidelity was not affected by its particular floral or other traits. In contrast, insect species varied significantly in constancy (“insect”: $F_{5, 3919} = 2.29$, $P = 0.04$). Specifically, *Sphex pennsylvanicus* wasps were less likely to fly between conspecifics than honeybees (*Apis mellifera*; Fig. 2C, $P < 0.05$, Tukey); other insects did not vary significantly ($P > 0.05$, Tukey). The constancy of visitors also depended on the plant taxon visited (Fig. 2C, “insect $\times$ plant”: $F_{10, 3919} = 13.72$, $P < 0.0001$). These patterns suggested that visitors distinguished one plant species from the others. For example, honeybees were more likely to move conspecifically when foraging on *Asclepias syriaca*, lepidoptera had the highest constancy on *A. incarnata*, and *S. ichneumoneus* had the highest constancy on *A. verticillata* (Fig. 2C, Tukey, $P < 0.05$). In contrast, *Xylocopa virginica* were more likely to move to another taxon while foraging on *A. verticillata* (Fig. 2C, Tukey, $P < 0.05$).

POLLINATOR EFFECTIVENESS AND EFFICIENCY: CAPTURE DATA

We collected a total of 722 insects from 15 populations over three years. Insect pollinators varied significantly (Kruskal-Wallis, $P < 0.05$, $df = 19$; Fig. 3) in the number of pollinia carried (vector pollen load). *Apis mellifera* carried more pollinia of *Asclepias incarnata* and *A. verticillata* than of *A. syriaca* ($\chi^2 = 77.1$, $df = 2$, $P < 0.001$; Fig. 3), as did *Bombus* species ($\chi^2 = 81.1$, $df = 2$, $P < 0.001$), *Xylocopa virginica* ($\chi^2 = 10.1$, $df = 1$, $P < 0.001$), sphecids

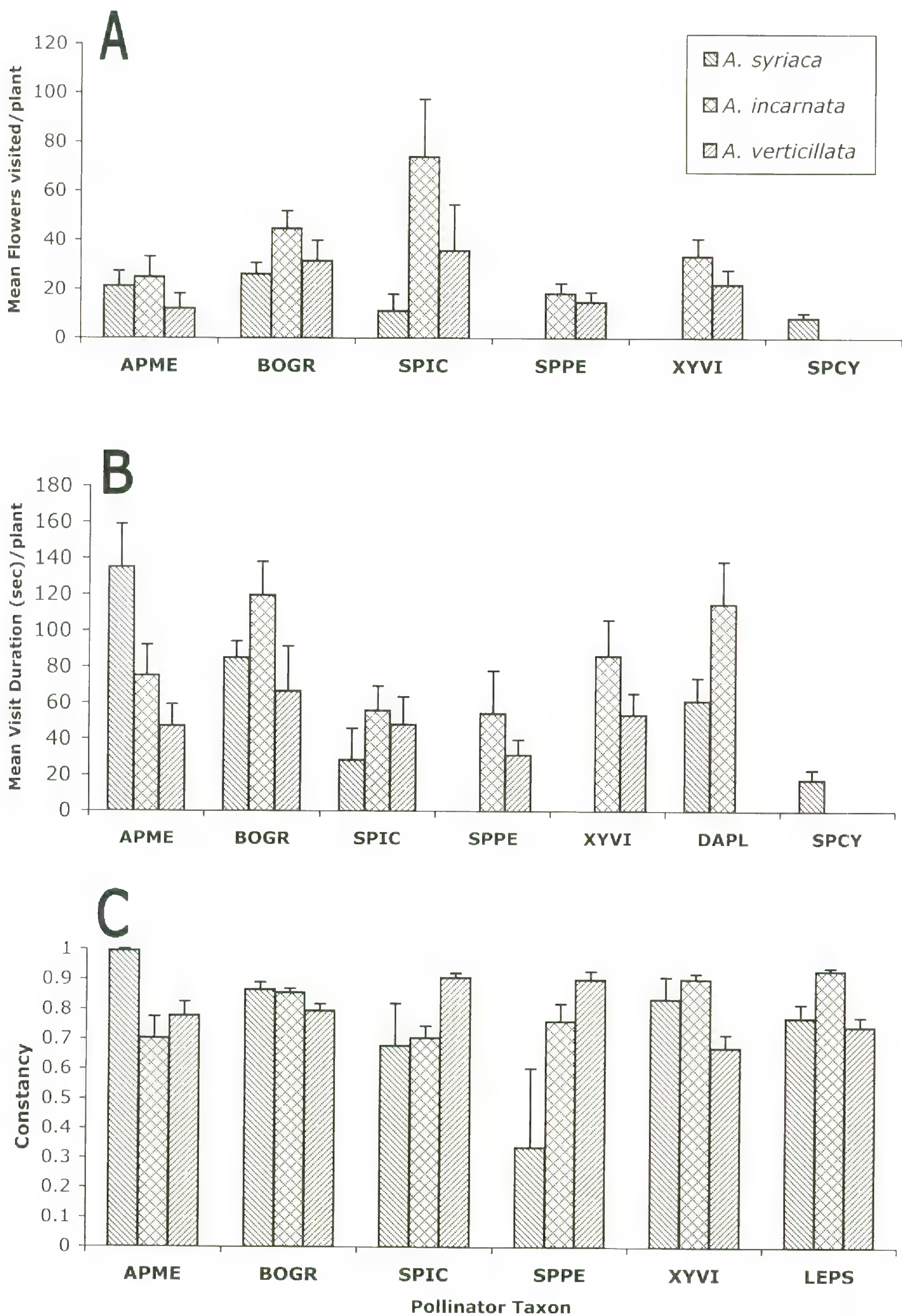


Figure 2. —A. Mean number of flowers visited per plant by the major pollinators of three *Asclepias* species growing in a common garden. —B. Mean visit duration per plant of major pollinators to three *Asclepias* species growing in a common garden. —C. Mean proportion of flights to conspecific plants (constancy) by insects foraging on three *Asclepias* species growing in a common garden. All error bars represent 1 SEM. Insect taxon codes: APME = *Apis mellifera*; BOGR = *Bombus griseocollis*; DAPL = *Danaus plexippus*; LEPS = Lepidopterans; SPCY = *Speyeria cybde*; SPIC = *Sphex ichneumoneus*; SPPE = *Sphex pennsylvanicus*; XYVI = *Xylocopa virginica*.

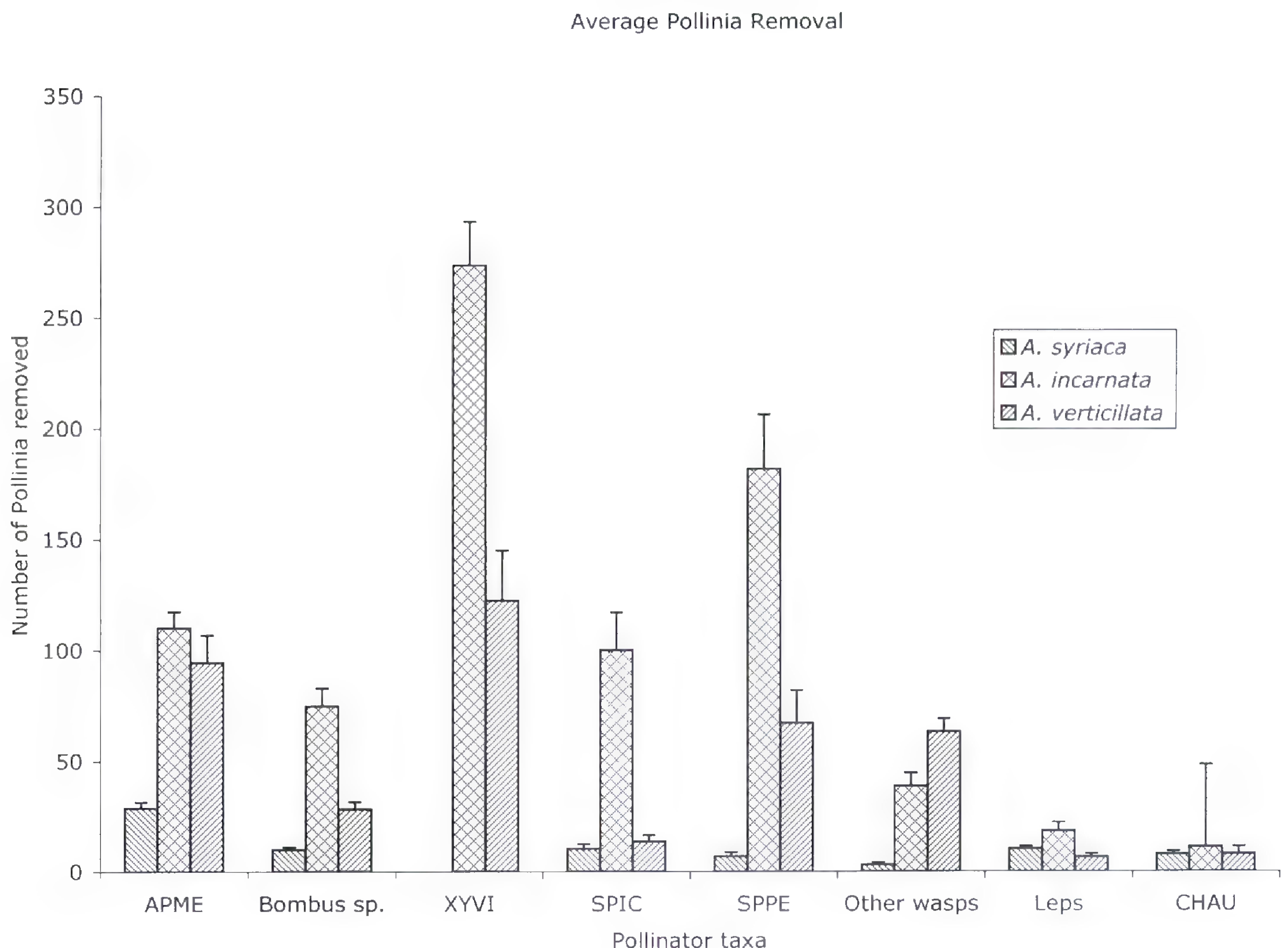


Figure 3. Estimated pollinium loads removed by insect pollinators based on the numbers of corpuscula and pollinia attached to captured visitors of three *Asclepias* species in common garden plots and natural populations. Error bars represent 1 SEM. Insect taxon codes: APME = *Apis mellifera*; CHAU = *Chaulignathus* species; Leps = Lepidopterans; SPIC = *Sphex ichneumoneus*; SPPE = *Sphex pennsylvanicus*; XYVI = *Xylocopa virginica*.

wasps (*S. ichneumoneus* and *S. pennsylvanicus*; $\chi^2 = 42.7$, $df = 2$, $P < 0.001$), and other wasps (*Myzinum* species, etc.; $\chi^2 = 16.1$, $df = 2$, $P < 0.001$). We combined all *Bombus* species to increase the total sample sizes, with *B. griseocollis* being the most common (73/97 on *A. incarnata*; 33/60 on *A. syriaca*; 39/44 on *A. verticillata*). *Xylocopa virginica* carried more pollinia of *A. incarnata* than any other taxon sampled ($P < 0.01$, $df = 6$) and more pollinia of *A. verticillata* than any insect except for *A. mellifera* ($P < 0.05$, $df = 6$). The detailed distribution of pollinia on insect bodies is provided in Kephart and Theiss (2004: table 4).

We also graphically compared estimates of the effectiveness of insects at removing pollinia (number carried corporally) with estimates of the efficiency of insect vectors in transferring pollinia to flowers (Fig. 4). The ordination of both variables in two dimensions shows several patterns evident in capture data: (1) high putative pollinium transfer efficiencies (i.e., estimated insertion effectiveness) for sphecids wasps; (2) relatively low probabilities for the potential insertion of *Asclepias verticillata* pollinia into later-

visited flowers; (3) higher estimated insertion probability for diurnal lepidopterans on *A. incarnata* relative to its congeners; and (4) typically low numbers of pollinia on the bodies of insects foraging on *A. syriaca* relative to *A. incarnata* and *A. verticillata* (Fig. 4).

SINGLE VISIT EFFECTIVENESS

In both 2004 and 2005, we observed relatively few visits to *Asclepias* across all pollinator taxa (Table 1). Due to these low sample sizes and large standard errors, we did not attempt statistical analyses. *Bombus griseocollis* had the highest mean removal rate on *A. incarnata* (0.71 ± 0.35), and the day-flying hawkmoth (*Haemorrhagia diffinis*) had the lowest removal rate (*A. syriaca*, 0 ± 0 , $N = 3$; *A. incarnata*, 0.03 ± 0.06 ; Table 1). We observed a small subset of taxa inserting pollinia (Table 1), but these events were rare.

MULTIPLE INDICES OF POLLINATOR EFFECTIVENESS

Our indices of pollinator effectiveness comprise two broad categories: (1) pre-visit measures (visitation

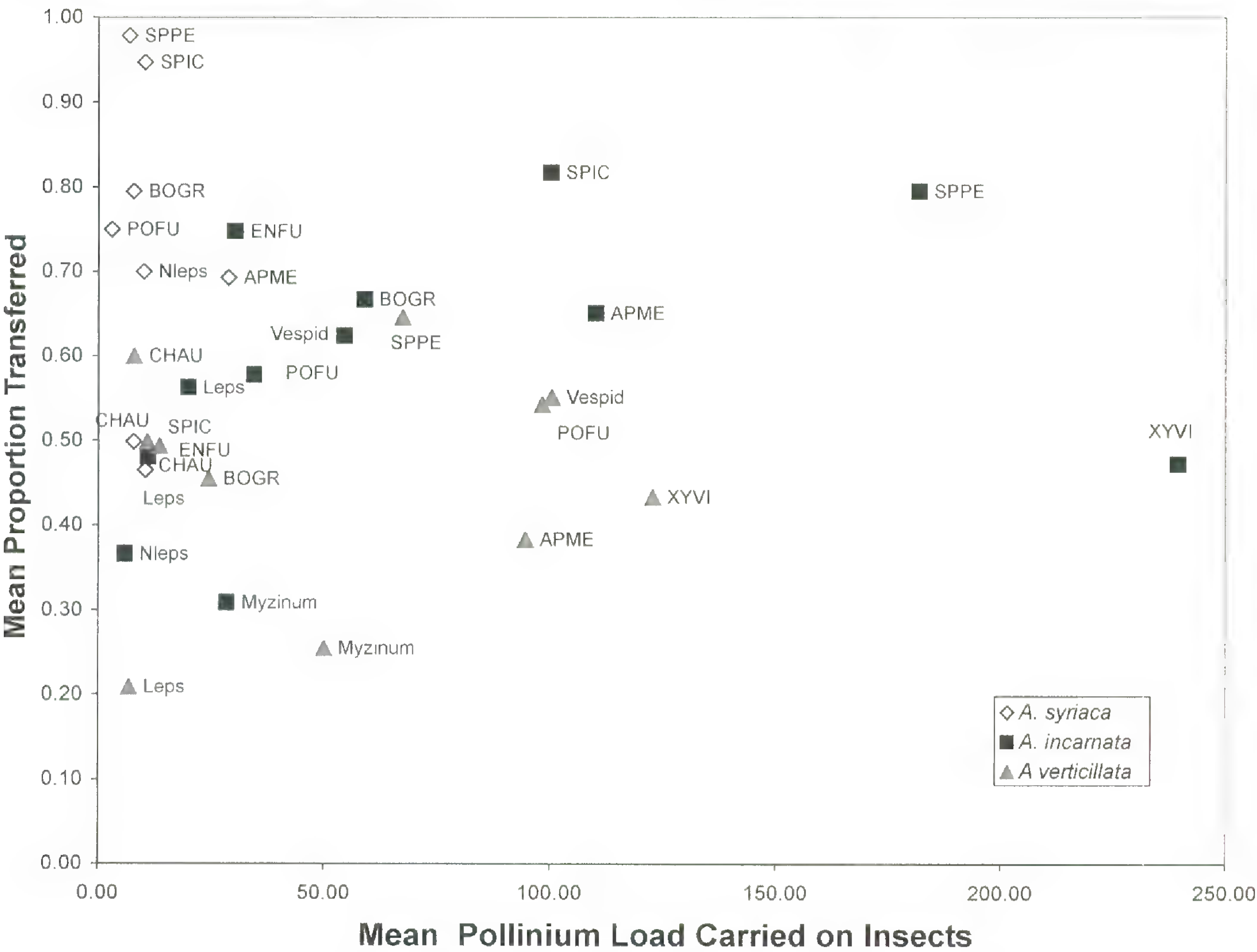


Figure 4. Ordination of mean pollinium load on captured insects relative to the estimated proportion of pollinia transferred from these same insects, presumably primarily to later-visited flowers (i.e., proportion missing from the total of all attached corpuscula $\times$ 2). Insect taxon codes: APME = *Apis mellifera*; BOGR = *Bombus griseocollis*; CHAU = *Chaulignathus* species; ENFU = *Entypus fulvicornis*; Leps = Lepidopterans; Myzinum = *Myzinum* species; Nleps = Nocturnal lepidopterans; POFU = *Polistes fuscatus*; SPIC = *Sphex ichneumoneus*; SPPE = *Sphex pennsylvanicus*; Vespid = Vespid wasps; XYVI = *Xylocopa virginica*.

Table 1. Comparisons of visitors to *Asclepias* species on experimental virgin flowers in natural populations. Numbers in parentheses following focal visitor species are the numbers of individuals observed. Pollinium removals (insertions) = mean number of pollinia removed (inserted) per flower visited (1 SEM); efficiency = mean number of insertions per removal (1 SEM).

Plant taxa	Insect taxa	Pollinium removals	Pollinium insertions	Efficiency
<i>Asclepias syriaca</i>	<i>Apis mellifera</i> (5)	0.253 (0.045)	0.051 (0.032)	0.200 (0.122)
	<i>Haemorrhagia diffinis</i> (3)	0	0	0
	<i>Myzinum</i> spp. (1)	0.364	0	0
<i>A. incarnata</i>	<i>Apis mellifera</i> (2)	0.125 (0.125)	0	0
	<i>Bombus griseocollis</i> (4)	0.708 (0.177)	0	0
	<i>Haemorrhagia diffinis</i> (3)	0.033 (0.033)	0	0
	<i>Myzinum</i> spp. (7)	0.465 (0.299)	0.042 (0.039)	0.108 (0.091)
	<i>Polistes fuscatus</i> (2)	0.395 (0.395)	0	0
	<i>Sphex ichneumoneus</i> (3)	0.164 (0.056)	0	0
	<i>Sphex pennsylvanicus</i> (12)	0.389 (0.664)	0.036 (0.019)	0.056 (0.028)
<i>A. verticillata</i>	<i>Xylocopa virginica</i> (3)	0.386 (0.122)	0.037 (0.037)	0.077 (0.077)
	<i>Bombus</i> spp. (5)	0.164 (0.065)	0.006 (0.006)	0.050 (0.050)
	<i>Myzinum</i> spp. (4)	0.357 (0.267)	0.119 (0.089)	0.490 (0.187)
	<i>Sphex ichneumoneus</i> (1)	0	0	0
	<i>Sphex pennsylvanicus</i> (4)	0.125 (0.066)	0	0

rate, visitation time, flowers visited, and constancy) and (2) post-visit measures (pollinium load, proportion inserted, and single visit effectiveness). Not surprisingly, varied measures yielded slightly different rank orders of pollinator taxa on each *Asclepias* species, with lepidopterans ranked as least effective overall (Table 2). Bees, including native *Bombus* species, ranked highest in pre-visit measures, especially on *A. syriaca* and *A. incarnata*, whereas wasps garnered greater importance in post-visit measures. Despite lower constancies on *A. incarnata* and *A. syriaca*, sphecids exhibited high pre- and post-visit effectiveness. Non-native honeybees were most effective on *A. syriaca* and carried higher numbers of its pollinaria than some more common visitors (Fig. 3, Table 2).

DISCUSSION

The evolution of floral divergence is often linked to specialization for particularly “effective” pollinators (sensu Stebbins, 1970), yet few investigations (e.g., McIntosh, 2005; Rymer et al., 2005) use multiple measures to compare the efficiency and effectiveness of pollinators foraging on closely related species growing in the same community. As in other studies (Willson et al., 1979; Jennersten & Morse, 1991; Ivey et al., 2003; Kephart & Theiss, 2004), a broad spectrum of hymenopteran pollinators dominated the visitor fauna of all three milkweeds, ranging in size and type from small-bodied honeybees to large native bees and sphecids or vespids wasps. Of diurnal lepidopterans, nymphalids and papilionids were more prevalent than pierids, hesperids, and hawkmoths, yet none were frequent across the entire flowering season. Except in constancy and visit duration on *Asclepias incarnata*, lepidopterans ranked low in pre- and post-visit indices for all taxa.

One important goal of this study was to identify whether the effectiveness or behavior of generalist pollinators differed on these co-occurring *Asclepias*, which vary for traits that include height, flower size, scent, and color. Pollinators differed in their approach and primary body contact on these diverse milkweeds (Macior, 1965; Kephart & Theiss, 2004), and this study extends these findings to vector pollen loads and estimated efficiencies of pollinium transfer. First, major pollinators varied markedly in the number of pollinia carried, with means ranging from 10 or fewer for hymenopterans and lepidopterans on *A. syriaca* to more than 200 for carpenter bees and nearly as many for sphecids wasps on *A. incarnata*. Vector pollinium load did not vary strictly with floral size; however, the number of corpuscula of *A. incarnata* on captured insects significantly exceeded that of both smaller-

flowered *A. verticillata* and larger-flowered *A. syriaca* (Fig. 3). Despite small samples, independent estimates of pollinium removal rates from virgin flowers also were highest in *A. incarnata* after single visits by all four insect taxa for which pairwise comparisons between plant species were possible (*Apis mellifera*, *Bombus* species, *Sphex* species, and *Myzinum* species; Table 1). These results are also consistent with the high frequency of foraging bouts detected on *A. incarnata*.

Second, as evident from the ordination of estimates of pollinia removal and insertions (Fig. 4), pollinators visiting *Asclepias syriaca* overall had a higher proportion of pollinia missing from the attached corpusculum of each pollinarium. If pollinia from each species are lost at the same rate through grooming and other behaviors, these data suggest that while pollinators may remove fewer pollinia from *A. syriaca* than other sympatric asclepiads, they are more efficient at inserting them into subsequently visited flowers relative to the other two species. This potential for increased effectiveness may be associated with the position of attachment: the pollinia of *A. syriaca* attach preferentially to the aroliar pad located between the claws of all major pollinators while the tarsal hairs are the primary attachment sites for *A. incarnata* and *A. verticillata* (Morse, 1985; Kephart & Theiss, 2004; for *A. solanoana* Woodson, Lynch, 1977). The differential positioning of pollinia, combined with the apparent increased efficiency of insertion of *A. syriaca* pollinia, implies the potential for niche partitioning of pollinium attachment sites on the vector body. Studies of orchids, which also have pollinia, suggest that species sharing a common pollinator differentially place pollinia on multiple parts of the insect body, thereby maximizing pollinator effectiveness and minimizing hybridization (Nilsson et al., 1987; van der Pijl & Dodson, 1966). A broader survey of asclepiads would allow exploration of the mechanistic factors that facilitate the aroliar attachments of the larger *A. syriaca* pollinia.

We also studied behaviors that potentially influence pollinator effectiveness, including the per-flower visitation rate (i.e., related to handling time from a foraging perspective), pollinator constancy (sensu Gregear & Laverty, 2001), and two measures potentially affecting geitonogamy (the duration of visits to plants and the number of plants visited per foraging bout). The large number of *Asclepias incarnata* flowers visited/time for *Bombus* species, *Sphex* species, and *Xylocopa virginica*, relative to other flower types, is consistent with the high pollinaria loads of *A. incarnata* on insects and a higher estimated effectiveness by insects in removing its pollinia from virgin flowers. Another explanation yet

Table 2. Taxa placed in rank order for each measure of effectiveness. Relative importance = product of pollinator frequency and insect density/plot as calculated in Kephart and Theiss (2004); visitation time = mean time spent per plant (Fig. 2B); no. of flowers visited = mean number of flowers visited (Fig. 2A); constancy data based on Figure 2C; pollinium removal = estimate of pollinia removed based on counts of corpuscula on captured insects (Fig. 3); proportion inserted obtained from Figure 4; single-visit data derived from Table 1. Insect taxon codes as in Figure 4. HADI = *Haemorrhagia diffinis*.

Plant taxa	Pre-visit measures of pollinator effectiveness				Post-visit measures of pollinator effectiveness		
	Relative importance	Visitation time	No. of flowers visited	Constancy	Pollinium removal	Proportion inserted	Single visit
<i>Asclepias syriaca</i>	<i>Bombus</i> spp.	APME	<i>Bombus</i> spp.	APME	APME	SPPE	<i>Myzinum</i> spp.
	APME	<i>Bombus</i> spp.	APME	<i>Bombus</i> spp.	SPIC	SPIC	APME
	Leps	DAPL	SPIC	XYVI	<i>Bombus</i> spp.	<i>Bombus</i> spp.	HADI
	All wasps	SPIC	SPCY	Leps	Leps	Nocturnal Leps	
		Leps		SPIC	<i>Chaulignathus</i> spp.	APME	
				SPPE	SPPE	<i>Chaulignathus</i> spp.	
<i>A. incarnata</i>					Non-sphecid wasps	Leps	
	<i>Bombus</i> spp.	<i>Bombus</i> spp.	SPIC	Leps	XYVI	SPIC	<i>Bombus</i> spp.
	XYVI	DAPL	<i>Bombus</i> spp.	XYVI	SPPE	SPPE	<i>Myzinum</i> spp.
	Leps	XYVI	XYVI	<i>Bombus</i>	APME	APME	POFU
	All wasps	APME	APME	SPPE	SPIC	<i>Bombus</i> spp.	SPPE
	APME	SPIC	SPPE	APME	<i>Bombus</i> spp.	Leps	XYVI
<i>A. verticillata</i>				SPIC	Non-sphecid wasps	Non-sphecid wasps	SPIC
					Leps	<i>Chaulignathus</i> spp.	APME
					<i>Chaulignathus</i> spp.	XYVI	HADI
	All wasps	<i>Bombus</i> spp.	SPIC	SPIC	XYVI	SPPE	<i>Myzinum</i> spp.
	<i>Bombus</i> spp.	XYVI	<i>Bombus</i> spp.	SPPE	APME	<i>Chaulignathus</i> spp.	<i>Bombus</i> spp.
	APME	SPIC	XYVI	<i>Bombus</i> spp.	SPPE	SPIC	SPPE
	Leps	APME	SPPE	APME	Non-sphecid wasps	XYVI	SPIC
	XYVI	SPPE	APME	Leps	<i>Bombus</i> spp.	<i>Bombus</i> spp.	
				XYVI	SPIC	Non-sphecid wasps	
					<i>Chaulignathus</i> spp.	APME	
					Leps	<i>Myzinum</i> spp.	
						Leps	

to be tested is that the small flowers of *A. incarnata* relative to *A. syriaca* may allow more contact points/flower visit and, hence, more pollinium removals. Its vertical umbel position during insect visits versus the flexible *A. verticillata* pedicels and lax peduncles of *A. syriaca* may have a similar effect. The relatively flat-topped, upright umbels or other floral cues may also influence the high prevalence, constancy, and duration times of large lepidopterans (e.g., *Danaus*, *Papilio*, *Battus*) on *A. incarnata*. Long foraging times for non-native honeybees on *A. syriaca* relative to native pollinators further suggest that geitonogamous selfing may reduce the effectiveness of *Apis mellifera*. Similarly, the number of *A. incarnata* plants visited per bout by honeybees averaged 0.25–0.5 times that of native *Bombus*, *Xylocopa*, and both nymphalid and papilionid butterflies, corroborating the high potential for geitonogamy detected by Ivey et al. (2003) for *A. mellifera* relative to other pollinators.

Pollinators varied with respect to constancy, but the idiosyncratic nature of variation in constancy among plant species suggests that species-specific floral traits do not consistently induce fidelity. In contrast, different insect taxa varied significantly in their probabilities of moving conspecifically when foraging, and this behavior varied seasonally and with the plant species visited (Fig. 2C). Annual variation in insect emergence and flowering phenology (e.g., Wiggam & Ferguson, 2005) can potentially influence the relative abundance or efficiency of pollinators over the course of a season or between years. For all milkweeds, constancy was highest during peak flowering, when pollinators are primarily foraging within a species. This increases potential intraspecific pollen transfer while maximizing resource gains to insects by decreasing handling time (Slaa & Biesmeijer, 2005). In all, further research is essential to understand the dynamics of foraging variability in multispecies assemblages and its effect on pollination efficiency, e.g., the near absence of *Xylocopa virginica* on *Asclepias syriaca* likely reflects its late season emergence, but this requires more intensive study across years.

A third goal of our study was to compare several commonly used pre- and post-visit indices of pollinator effectiveness across co-occurring plant populations. We especially sought to compare major pollinators for two widely used measures: (1) pollen load on captured insects, and (2) pollen removal from anthers and deposition on stigmas after single visits of insect pollinators to virgin umbels. In both measures, non-target variables that may influence the outcome-dependent variables are not fully controlled. Vector pollen loads are potentially affected by the insect age and foraging time. The pollinium load on a captured

insect is not a direct evaluation of pollinium removal or insertion and is confounded by pollinium loss due to insect grooming or other behaviors (Lynch, 1977; Morse, 1985). Similar challenges exist for single-visit data using virgin umbels. Although some investigators inspect pollinators after a floral visit (Lynch, 1977; Morse, 1985; Fishbein & Venable, 1996; Ivey et al., 2003), most researchers find it highly impractical to measure the pollen levels on an animal's body prior to floral visitation. This problem has been little discussed in the literature (Morse, 1985), yet a high prior pollen load (e.g., from an insect that has foraged all morning vs. a newly emerged insect) likely affects the probability of pollen deposition on virgin flowers. Some of the bias involved in capture data should be diminished by a large sample size or by observing pollinium removal and insertion after single visits to virgin flowers. Although we found some congruence in these measures, low visitation rates in the populations used for single-visit analyses and shifts in weather and mowing schedules on sampling dates prevented a more robust comparison of effectiveness based on capture data and single-visit removal rates.

We detected variation in pollinator fidelity, pollinia removal, and pollinator efficiency among sympatric species of *Asclepias*. Overall, pollinators were more efficient in transferring pollinia of *A. syriaca*, although they carried more pollinia of *A. incarnata*. Native and non-native bees performed similarly across taxa, although the non-native honeybee, *Apis mellifera*, tended to be inefficient in transferring pollinia even after high removal rates (Table 2). Further exploration of the correlations among pre- and post-contact measures of pollinator effectiveness as well as designing of experiments to evaluate the potential associations of lepidopterans and hymenopterans with effective pollination of *Asclepias* species that differ in floral traits would be beneficial to our understanding of this system. Overall, we know remarkably little about how generalized pollination systems affect floral divergence and about the effect of spatio-temporal variation in pollinators on sympatric populations of plants with differing floral cues or architectures.

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